Entrainment of bacterial synthetic clocks.

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***Abstract* — We force a self-sustained synthetic gene oscillator in growing colonies of *E. coli* cells, exploring a wide range of forcing amplitudes and periods. From our single-cell data, we are able to determine the synchronization region in the amplitude-period parameter space. For parameters in this region, individual trajectories are phase and frequency locked to the environmental stimulus. A detailed stochastic model of the gene oscillator is being developed to account for our experimental observations.**

***Keywords* — Key words or phrases in order of importance, separated by commas. Key words are optional. Use no more than three lines.**

I. Background

Quite often biological oscillators become synchronized to environmental cues. A paradigmatic case is found in the circadian rhythms in animals, plants and some bacteria, that become entrained to the periodic 24 hour cycle of light and darkness [3].

In an effort to understand the fundamental mechanisms of biological rhythms, our lab has adopted a synthetic biology approach and a tunable and robust genetic oscillator in single *E. coli* cells was developed [1]. This oscillator is self-sustained, tunable and robust, but as cells divide the oscillations become desynchronized within a few generations. In analogy with the environmental driving of circadian clocks, we force this bacterial oscillator to study its dynamics. In our case the driving signal is a harmonically modulated concentration of Arabinose. This saccharide molecule acts on the genetic construct as an activator of transcription. We find that the double feedback oscillator can become phase and period locked to the external input, allowing us to define a synchronization region (Arnold tongue [4]) in the two dimensional amplitude-period space.

II. Experiments and Results

To perform the forcing experiments we developed a novel microfluidic device that permits the observation of multiple colonies of bacterial cells for at least 150 generations while being subjected to an external signal. We scanned multiple frequencies and amplitudes of forcing. The images from more than 30 time-lapse experiments were analyzed using a custom made software for single cell tracking. In this way we obtained reliable statistical distributions for the period of single cell oscillations and for the phase difference between cell oscillations and the external signal.

Although the synchronization of individual oscillators to the external stimulus is evident from the experimental time-lapse movies, period and phase difference distributions show quantitatively, for each tested forcing strength, the interval of periods where phase and frequency locking occurs. When entrainment is at work, the single-cell period distribution displays one or more peaks centered around a multiple of the forcing period, while the corresponding phase difference distribution is single peaked. Outside the synchronization region, the period of oscillations tends towards the natural period of the oscillator, the phase difference distribution becomes uniform for short forcing periods and multimodal for long ones.

Based on our quantitative measurements, we are developing a detailed stochastic model to describe the observed dynamics.

III. Conclusion

Through the combination of automated single-cell fluorescence microscopy, microfluidics and automated image analysis we performed a quantitative experimental study of the forced dynamics of a self-sustained synthetic genetic oscillator. Our work provides the first experimental demonstration that synthetic genetic oscillators can be entrained by external cues.

References

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